#### Figure

Changed

Primer R

↑ pase¢↑

CACCGCTTGCCCCCAGAATGGATGCGCATGTCTG
CACCGCTTGCCCCCAGAATGGAGGGGGGTGTCTG
TATTACTGGGCGAGGTGTCCT. ........GTGGCGAACGGGGGTCTTACCTCCCACAGACATAA¥GACCCGCTCCACAGGA..

Target Sequence

**Polymorphic** 

nucleotide

PCR amplify

Fok I/Fsp I

GAACGGGGGTCTTACCTCCCACAGACATAATGACCCGCTCCACAGGA. CTTGCCCCCAGAATGGATGCGCATGT¢TG7ATTACTGGGCGAGGTGTCCT...

Digest with Fok I and Fsp I

GCATGTCT 8 Mer GCATGGGCGAGGTGTCCT... CCCACAGACATA ATGACCCGCTCCACAGGA.. CTTGCCCCCAGAATGGATGC GAACGGGGGTCTTACCTCCT

#### Figure 2

Cut with Fok I

Fsp I

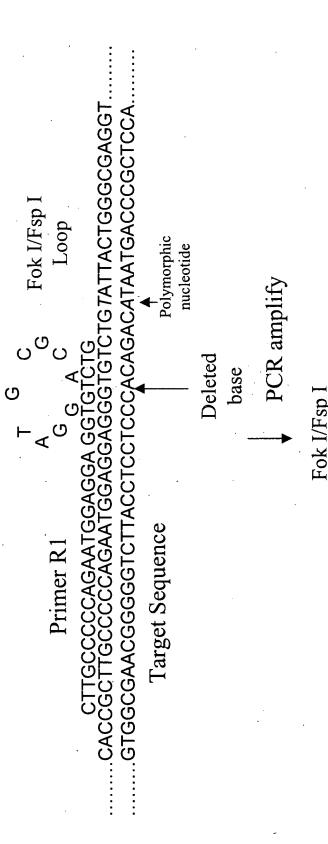
↑ nnnnnnTGCGCÅnnnnn nnnnnACGCG∓nnnnnn Cut with Fsp I

nnnnnnTGC GCAnnnnnn nnnnnnACG CGTnnnnn

Fok I GGATG CCGAC TGCGCA Fsp I ACGCGT Combined Fok I and Fsp I site

GGATGCGCA Fok I/Fsp I CCGACGCGT

## Cossor espectation Figure 4

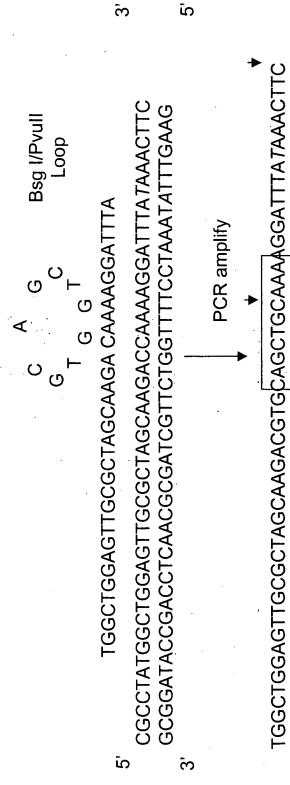


GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA. Digest with Fok I and Fsp I

CTTGCCCCCAGAATGGAGGAGGATGCGCAGGT&TCTG1ATTACTGGGCGAGGT.

CTTGCCCCCAGAATGGAGGAGGATGC GCAGGTGT CTG7ATTACTGGGCGAGGT. CGTCCACAGACA TAATGACCCGCTCCA... GAACGGGGTCTTACCTCCTCCTACG

#### posmor emorano Figure 5



ACCGACCTCAACGCGATCGTTCTGCACGTCGACGTTTTCCTAAATATTTGAAG

Digest with Bsg I and Pvull

CTGCAAAAGGATTTAT AAACTTC GACGTTTTCCTAAA TATTTGAAG 16 mer TGGCTGGAGTTGCGCTAGCAAGACGTGCAG ACCGACCTCAACGCGATCGTTCTGCACGTC

#### Figure 6

Ω က် Pvull/Fok | CGCCTATGGCTGGAGTTGCGCTAGCAAGGACCAAAAGGATTTATAAACTTC GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAATATTTGAAG тевствеваттесеставсавасса абеватта PCR amplify ũ ŝ

CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAGC†GGATGAAGGA†TTA*T*AAACTTC GCGGATACCGACCTCAACGCGATCGTTCTGGTGTCGACCTACTTCCTAAATATTTGAAG

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Digest with Pvu II and Fok I

CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAG CTGGATGAAGGATTTA TAAACTTC GCGGATACCGACCTCAACGCGATCGTTCTGGTGTC GACCTACTTCCTAAATATTT GAAG 16 mer

### Figure 7

Fok I/Fsp I

GAACGGGGGTCTTACCTCCTCCT<mark>ACGCGTCCAC</mark>AGACATAATGACCCGCTCCA.... CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTG7ATTACTGGGCGAGGT.

Remove nucleotides and digest with Fok I

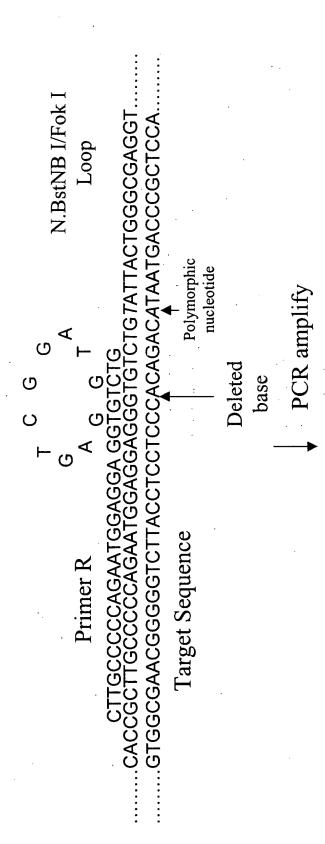
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGT GAACGGGGGTCTTACCTCCTACGCGTCCACAGACA Fill in with mass

Modified nucleotide

CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTmod GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA

Cleave with Bcg I

## Domination of the Bright of th

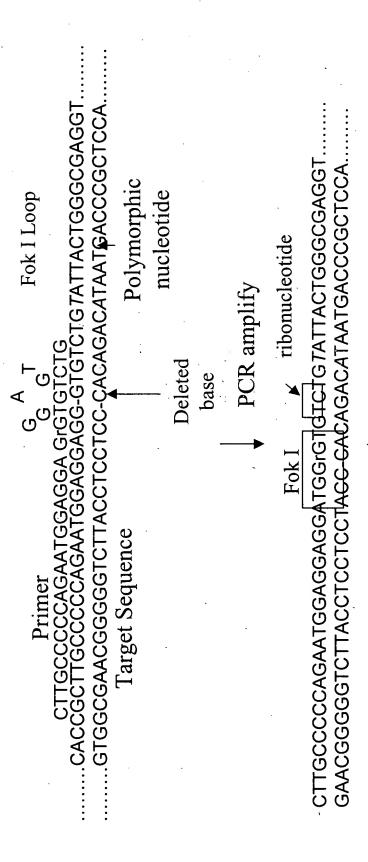


GAACGGGGGTCTTACCTCCTCTdAGCCTACCCACAGACATAATGACCCGCTCCA.. Digest with Fok I and N.BstNB CTTGCCCCCAGAATGGAGGAGA&TCGGATGGGTGTCTG*T*ATTACTGGGCGAGGT

N.BstNB I/Fok I

CTTGCCCCCAGAATGGAGGAGAGTCGGAT GGGTGTCTG $_{\it T}$  ATTACTGGGCGAGGT. GACCCGCTCCA.. GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAAT

#### Commor mercand Figure 10



7 mer CTTGCCCCCAGAATGGAGGAGGATGGrG TGTCTG7 ATTACTGGGCGAGGT GACCCGCTCCA. GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAAT

Digest with Fok I and

cleave with base

Figure 11. Methods for haplotyping based on physical allele separation

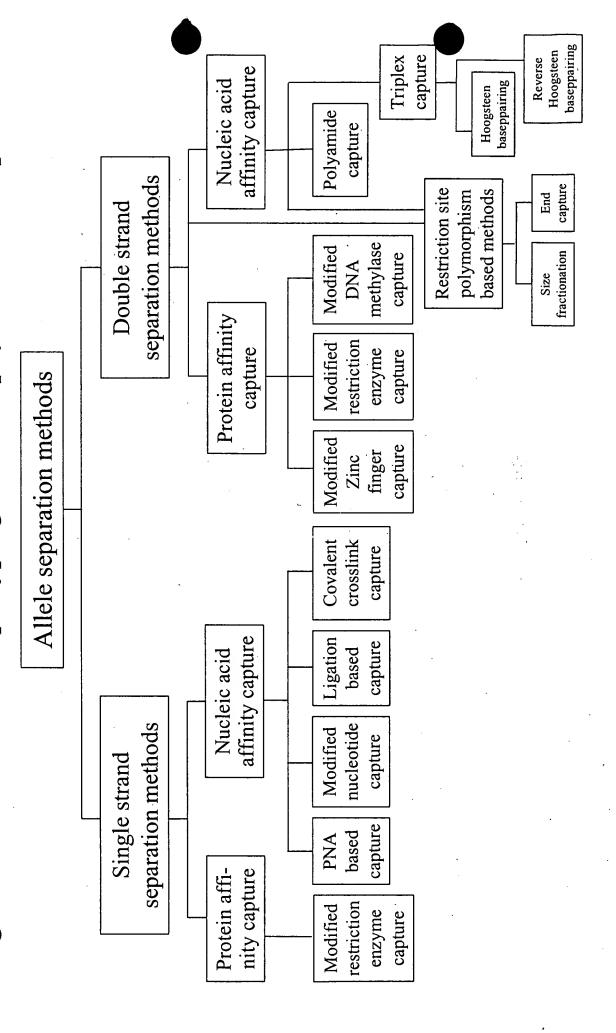


Figure 12. Methods for haplotyping based on allele specific amplification

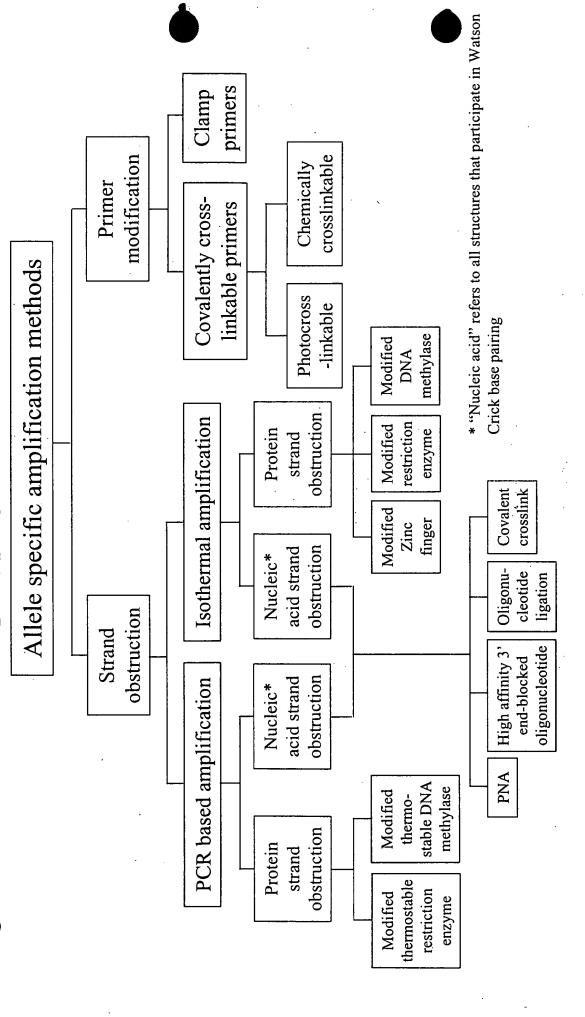
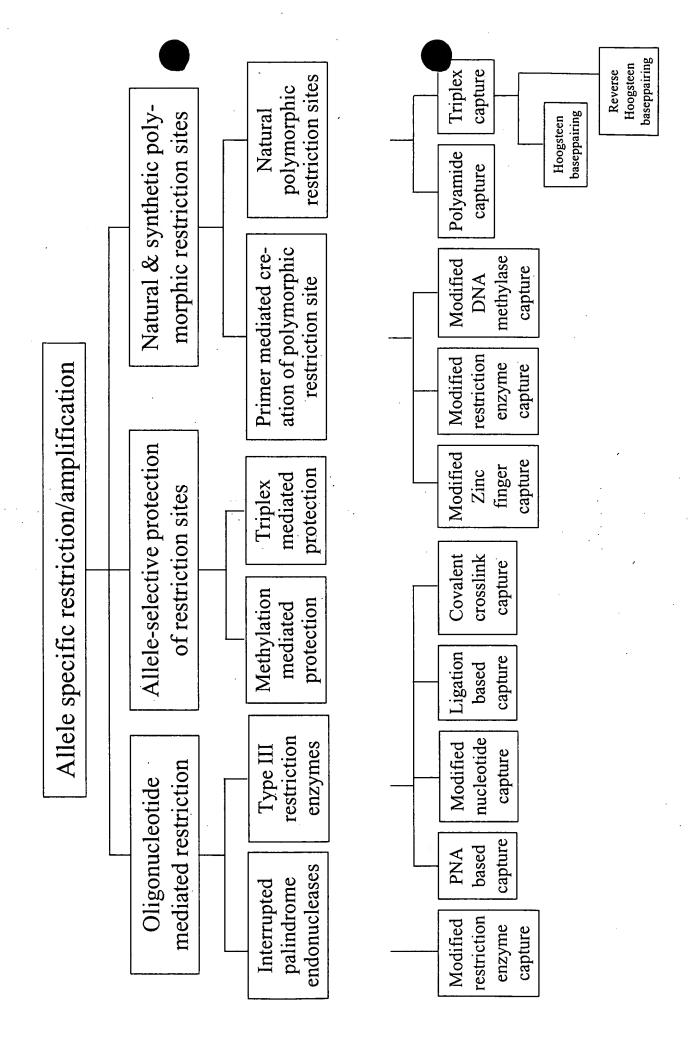


Figure 13. Methods for hablotybing based on allele specific restriction



D96970E8.10E500

# Figure 14: Hairpin PCR Primers

ALLELE 1  T  ALLELE 2  ALLELE 2  T PRIMER  T PRIMER
---

# Figure 15: Hairpin PCR Primers

ALLELE 1  C PRIMER  ATCCGGANNIN  TAGGCCTINININ	ATCCGGANNINININININININININININININININININI
ALLELE 2 C PRIMER	↓ PCR Amplify
ATCCGGANNNI	ATCCGGANNNNNNNNNNNTCCGGAT TAGGCCTNNNNNNNNNNNAGGCCTA

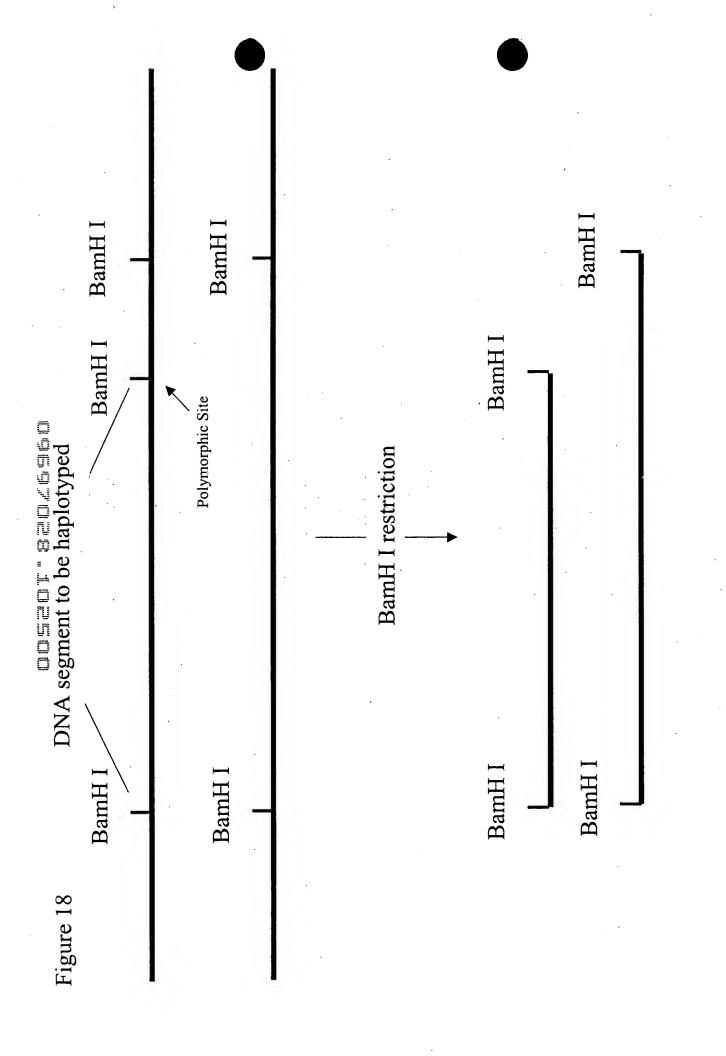
# Figure 16: Hairpin PCR Primers

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OCR of allele
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Minus strand resulting from PCR of
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——————————————————————————————————————	Hairpin loop forms inhibiting hybridization of PCR primer and amplification of allele 1  Minus Strand	PCR of allele 2	Minus Strand	mismatch allowing hybridization of PCR primer and amplification of allele 2 Minus Strand
		from	-	
TAGACCTNNNNNNNNNNAGGTCTA	N N TCCAGAT N AGGTCTA	Minus strand resulting from PCR of allele 2	TAGACCTNINININININININIAGGCCTA	N N N N TCC GAT N AGGCCTA N N N N N
TAGACCTN	ALLELE 1 T PRIMER		TAGACCTI	ALLELE 2 T PRIMER

# Figure 17: Hairpin PCR Primers

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Minus strand resulting from PCR of allele 1
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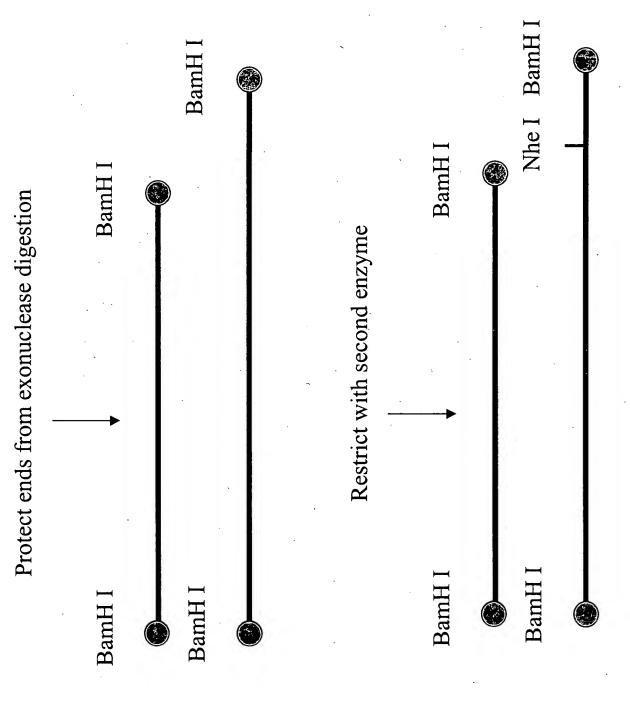


Figure 20

Digest with exonuclease

Add single strand nuclease to remove/degrade remaining single strand

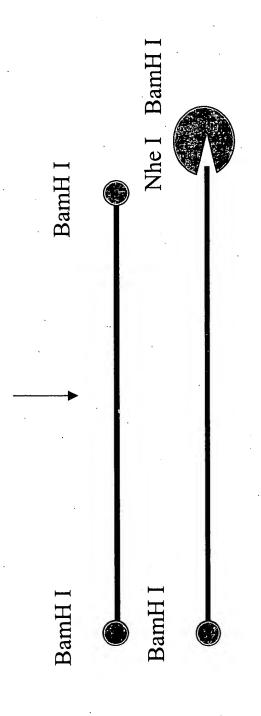
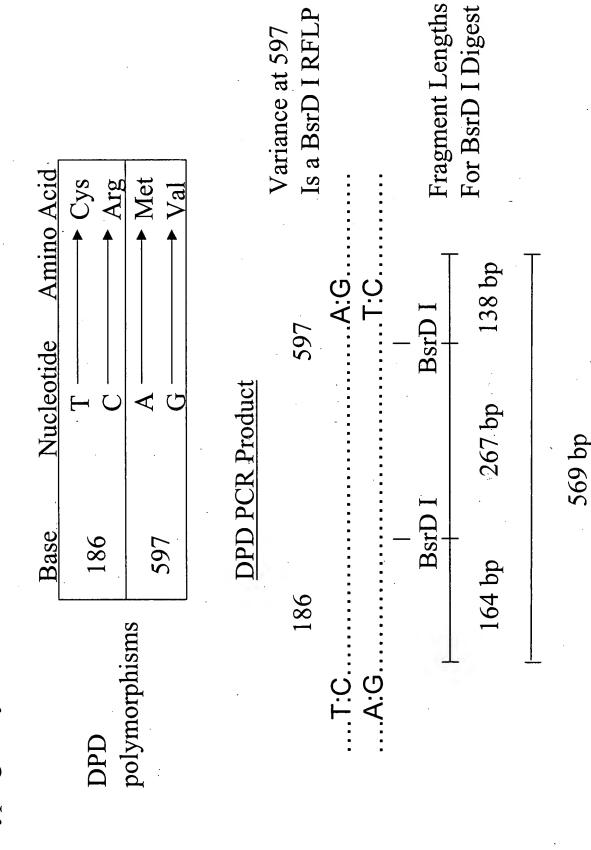


Figure 21. Dihydropyrimidine dehydrogenase (DPD) polymorphisms used in haplotyping assay.



, , ,	<u>acacag</u> actcatgcaactctg	5,0000000000000000000000000000000000000	actical actical acticity	5' actcatgcaactctg
	DPDASCF	THO A CLUCK	DPDASIF	DPDNSF
Α.		DPD	Primers	

~

DPD Sequence

<sup>5</sup>'actcatgcaactctg[T or C]gttccacttcggccaagaa , tgagtacgttgagac[A or G]caaggtgaagccggttctt <sub>3</sub>'

Z

## Figure 23. PCR Amplification Using DPDNSF Primer

5'actcatgcaactctg 	5'actcatgcaactctg 	5'actcatgcaactctgTgttccac	5, actcatgcaactctgCgttccac
DPDNSF primer	DPDNSF primer	T allele	C allele
Template: T allele	Template: C allele	PCR Product	PCR Product

# Figure 24. PCR Amplification Using DPDASTF Primer.

<sup>5</sup> ' acgcagactcatgcaactctg 3' 	5' <u>acgcagactcatgcaactctg</u> 	5, acq caqactcatgcaactctgTgttccac	5, acqcagactcatgcaactctgCgttccac
DPDASTF primer	DPDASTF primer	T allele	C allele
Template T allele	Template C allele	PCR Product	PCR Product

# Figure 25. PCR Amplification Using DPDASCF Primer

5' <u>acacag</u> actcatgcaactctg 3' 	5' <u>acacagactcatgcaactctg</u> 	5 acacagactcatgcaactctgTgttccac	<sup>5</sup> acacagactcatgcaactctgCgttccac
DPDASCF primer	DPDASCF primer	T allele	C allele
Template T allele	Template C allele	PCR Product	PCR Product

## COSTATE LORES

Figure 26. Hairpin Structures for PCR Products Generated Using DPDNSF Primer

Hairpin Structure T Allele Reverse Strand

> Hairpin Structure C Allele Reverse Strand

 $Tm = 40^{\circ}C$ 

Figure 27. Hairpin Structures for PCR Products Generated Using DPDASCF Primer

Hairpin Structure T Allele Reverse Strand

Hairpin Structure C Allele Reverse Strand

$$Tm = 42$$
°C

Figure 28. Hairpin Structures for PCR Products Generated Using DPDASTF Primer

Hairpin Structure T Allele Reverse Strand

$$Tm=42^{\circ}C$$

Hairpin Structure C Allele Reverse Strand

#### ALLELE C

### **DPDNSF** primer

actictg 
$$^{5}$$
 Tm =  $41^{\circ}$ C

$$Tm = 40^{\circ}C$$

$$Tm = 40^{\circ}C$$

$$Tm = 40^{\circ}C$$

3

#### ALLELE T

DPDNSF primer 
$$3'$$
 Tm =  $41^{\circ}$ C

#### ALLELE C

DPDASCF primer  $Tm = 60^{\circ}C$   $\frac{3'}{acacagactcatgcaactctg}$ 

- atgagt $\frac{3'}{|\ |\ |\ |}$   $T_m = 42^{\circ}C$  - cgttgagacGcaaggtg.....

Primer
Hybridization
and Amplification

#### ALLELE T

DPDASCF primer  $Tm = 60^{\circ}C$  acacagactcatgcaactctg

Hairpin inhibits
Primer Hybridization
and Amplification

5' <u>acacag</u>actcatgcaactctg

☐ atgagtctqtgt ☐☐☐☐☐☐☐☐☐☐☐☐☐☐ 5' — cgttgagacAcaaggtg......

#### ALLELE C

## DPDASTF primer Tm = 65°C

5' acqcagactcatgcaactctg

Hairpin inhibits
primer hybridization
and Amplification

5' acgcagactcatgcaactctg

| atgagtctg**c**qt | TTTT | | | | cgttgagacGcaaggtg......

#### ALLELE T

DPDASTF primer  $Tm = 65^{\circ}C$  acquaqactcatgcaactctg

– atgagtctqcqt  $^3$  Tm=2 – cgttgagacAcaaggtg....

Primer hybridizes and amplification ensues

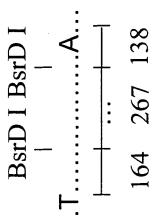
5,

Figure 32. Allele Specific Amplification of a Heterozygous Sample with Haplotype T<sup>186</sup>, A<sup>597</sup> and C<sup>186</sup>, G<sup>597</sup>

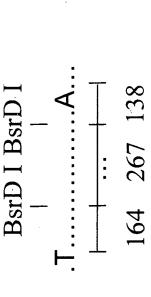
### **DPDNSF PRIMER**

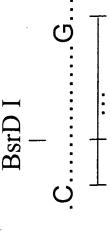
### DPDASTF PRIMER

### DPDASCF PRIMER



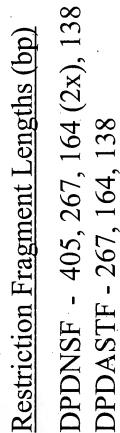
BsrD





405

164



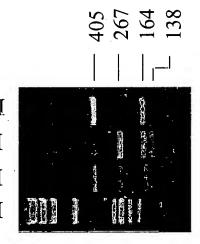
DPDASCF - 405, 164

405

164

Figure 33. BsrD I Digest of Allele Specific PCR Products.

DPDASCF DPDASTF PPDASTF PPDASCF



### Figure 34

Fok I/Fsp ] Loop ApoE21250-LR G CCCGGCTGGGCGCGGGCATG AGGACGTG

GCAGGCCCGGCTGGGCGCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGTACCGC CGTCCGGGCCGACCCGCGCCTGTACCTCCTGFACACGCCGGCGGACCACGTCATGGCG

Target Sequence

GGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCGCCT CCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGCGGA

Target Sequence

GGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG GGCTACTGGACGTCT CCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC

ApoE21250-LR

## OSSTABEL CEST

### Figure 35

T Allele Amplicon

CCCGGCTGGGCGCGGACATGCGCAAGGACGTGTGCGGCCGCCTGGTGCAGTAC GGGCCGACCCGCGCCTGTACCCTACGCGTTCCTGCACGCCCGGCGGACCACGTCATG CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCG GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGC

GGAGGTGGACGCGTTCGACGCATTCGCCGAGGGGGCGCTACGGCTACTGGACGTCTTCG CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC

#### C Allele Amplicon

CCCGGCTGGGCGCGGACATGGGATGCGCAAGGACGTGCGCGGCCGCCTGGTGCAGTAC GGGCCGACCCGCGCCTGTACCCTACGCGTACCTGCACGCGCCGGGCGGACCACGTCATG CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCG GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGC

GGAGGTGGACGCGTTCGACGCATTCGCCGAGGGGGCGCTACGGCTACTGGACGTCTTCG CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC